Molecular Epidemiologic Research on the Effects of Environmental Pollutants on the Fetus

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Evidence shows that fetuses and infants are more affected than adults by a variety of environmental toxicants because of differential exposure, physiologic immaturity, and a longer lifetime over which disease initiated in early life can develop. In this article we review data on the effects of *in utero* exposure to common environmental contaminants, including polycyclic aromatic hydrocarbons (PAH), particulate matter and environmental tobacco smoke (ETS). We then summarize results from our molecular epidemiologic study to assess risks from *in utero* exposures to ambient air pollution and ETS. This research study, conducted in Poland, used biomarkers to measure the internal and bioeffective dose of toxicants and individual susceptibility factors. The study included 160 mothers and 160 newborns. Ambient air pollution was significantly associated ($p \leq 0.05$) with the amount of PAH bound to DNA (PAH–DNA adducts) in both maternal and infant cord white blood cells (WBC). Newborns with elevated PAH–DNA adducts (greater than the median) had significantly decreased birth weight ($p = 0.05$), birth length ($p = 0.02$), and head circumference ($p = 0.0005$) compared to the newborns with lower adducts ($n = 135$). Maternal and infant cotinine levels were increased by active and passive cigarette smoke exposure of the mother ($p \leq 0.01$). An inverse correlation was seen between newborn plasma cotinine (nanograms per milliliter) and birth weight ($p = 0.0001$) and length ($p = 0.003$). Adducts were elevated in placental tissue and WBC of newborns who were heterozygous or homozygous for the cytochrome P4501A1 *MspI* restriction fragment length polymorphism (RFLP) compared to newborns without the RFLP. Levels of PAH–DNA and cotinine were higher in newborns than mothers. These results document that there is significant transplacental transfer of PAH and ETS constituents from mother to fetus; that PAH–DNA adduct levels in maternal and newborn WBC were increased with environmental exposure to PAH from ambient pollution; and that the fetus is more sensitive to genetic damage than the mother. The study also provided the first molecular evidence that transplacental PAH exposure to the fetus is compromising fetal development. If confirmed, these findings could have significant public health implications since a number of studies have found that reduction of head circumference at birth correlates with lower intelligence quotient as well as poorer cognitive functioning and school performance in childhood. *Key words*: air pollution, cigarette smoking, *CYP1A1 MspI* RFLP, GSTM1, newborns, PAH–DNA adducts, Poland. — *Environ Health Perspect* 107(suppl 3):451–460 (1999).
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The context of the work reviewed here is the growing awareness of the vulnerability of the developing fetus and child to certain environmental contaminants, including those commonly found in urban areas around the world. Following a summary of the toxicity and occurrence of polycyclic aromatic hydrocarbons (PAH), particulate matter (PM), and, environmental tobacco smoke (ETS), we discuss molecular epidemiology and biomarkers as research tools. We then review the results of research undertaken in Poland to assess risks from *in utero* exposures in newborns.

Enhanced Susceptibility of the Developing Fetus and Infant

Evidence indicates that fetuses and infants are more affected than adults by a variety of environmental toxicants because of differential exposure, physiologic immaturity, and a longer lifetime over which disease initiated in early life can develop. Experimental and human data indicate that the fetus and the young child are especially vulnerable to the toxic effects of PAH, ETS, PM, nitrosamines, pesticides, polychlorinated biphenyls (PCBs), and metals (*1–6*).

Several studies suggest that the fetus may be more prone to genetic damage and clears toxicants less efficiently than the adult. For example, given experimental evidence that the amount of PAH crossing the placenta and reaching the fetus is less than one-tenth of the dose to the mother (*7,8*), the levels of PAH–DNA adducts measured in rodent fetal tissue were higher than expected (*9,10*). As will be discussed, the same pattern was seen in Polish newborns (*11*). Increased adducts in the fetus relative to the adult could result from lower levels of phase II (detoxification) enzymes, decreased DNA repair efficiency in the fetus, or to lower fetal body fat, which sequesters some of the PAH in adults (*1,3,12*). In addition, a previous study reported higher levels of cotinine (a metabolite of nicotine) in fetal samples than in paired maternal samples (*13*), although others did not (*14,15*).

In particular, the developing nervous system is an extremely sensitive target (*16,17*). The central nervous system (CNS) and the peripheral nervous system both comprise highly specialized organs and tissues that are vulnerable to deficits in oxygen and nutrients and to damage by toxic chemicals. During the prenatal period, the developing fetal brain undergoes tremendous growth and differentiation as cells divide and migrate to form structures in many areas simultaneously. Neurotoxicants present in fetal circulation or tissues will have ready access to these activated cells, as the blood brain barrier does not develop until after birth. Because many brain areas are developing at the same time, prenatal exposures to toxicants might be expected to produce somewhat general effects on growth and development. Neurotoxicants

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would also be expected to interfere with the optimal development of the CNS structures if they are present during growth spurts in these structures. Examples of relatively well-characterized neurotoxicants affecting the fetus are lead, PCBs, and cocaine (*18–20*). The evidence for ETS is more limited (*21*). Circumstantial evidence regarding neurotoxicity of PAH is presented below.

Because brain development continues after birth, postnatal exposures to toxicants may be no less damaging than prenatal exposures but are likely to be more specific. For example, the organization of cortical, limbic, and cerebellar circuitry occurs postnatally and is sensitive to toxicant exposure. Also, the deposition of myelin and the proliferation of granule cells in the hippocampus are late developmental events that may be compromised by postnatal exposures. Susceptibility may depend upon the timing of the exposure with respect to the stages of brain development, the duration of the exposure, and the total amount of the exposure. Multiple exposures during early life may lead to cumulative neurobehavioral deficits.

The National Research Council and the U.S. Environmental Protection Agency have recognized the need for risk assessment and public health policy to pay special attention to protecting young infants and children from environmental toxicants (*1,6,22*). However, major gaps in knowledge have impeded the development of public health policy capable of effectively protecting the developing fetus and child. First, epidemiologic studies have been limited by the lack of accurate data on individual exposures and individual variation in response to toxicants (*23*). Second, there has been little effort to disentangle the influences of multiple environmental and susceptibility factors on growth and development. Research is needed to generate information on the magnitude of individual variation in exposure and susceptibility within this young population, as well as on the relationship between these factors and developmental impairment.

Domains of Fetal Growth and Development Likely to Be Affected by PAH, PM, and ETS Exposures

The most commonly measured parameters of fetal growth and development in the study of prenatal exposure to toxicants are head circumference, birth weight, birth length, and, to a lesser extent, size for gestational age (or growth retardation). Experimental and molecular epidemiologic studies have associated growth retardation with transplacental exposure to ETS and PAH. With respect to head circumference, most head growth occurs during the prenatal period, with more than 60% of adult head circumference attained at birth (*24*). The evidence that head circumference may be reduced by toxicant exposure comes largely from the prenatal alcohol and cocaine exposure literature. Some studies have linked prenatal cocaine exposure to a 1–2 cm reduction in infant head circumference (*18–20*). The literature suggests that reduction in infant head circumference at birth or during the first year of life is correlated with lower intelligence quotient (IQ) as well as poorer cognitive functioning and school performance in childhood (*20,25,26*). As will be discussed, we have found a significant association between elevated PAH–DNA adducts and a 0.9 cm decrement in head circumference of newborns (*27*). Others have shown that prenatal exposure to maternal tobacco smoke is associated with effects on cognitive development at age 3 years (*28*). Thus, the effect of prenatal exposure to PAH and ETS on cognitive development warrants further investigation.

Toxicity and Occurrence of Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons are among the best-characterized environmental toxicants. A number of PAH are reproductive and developmental toxicants, as well as mutagens and carcinogens. In addition to their ability to bind to and damage DNA, PAH such as benzo[*a*]pyrene (B[*a*]P) are capable of disrupting the endocrine system by altering metabolic pathways of natural hormones or otherwise interfering with their activity (*29,30*). Laboratory studies have found an association between transplacental exposure to certain PAH and adverse reproductive outcomes. Pregnant animals exposed to B[*a*]P and other PAH showed an increase in stillbirths, reabsorption and congenital abnormalities, and decreases in fetal weight (*29,31–36*). Subcutaneous administration of B[*a*]P to pregnant rats caused significant decreases in number of live offspring and in fetal weight, and significant increases in reabsorption sites ($p < 0.05$) (*29*). In the same experiment, B[*a*]P administration to pseudopregnant rats resulted in significant decreases in uterine weight. Similarly, gavage administration of coal liquefaction products containing high levels of PAH to pregnant Sprague-Dawley rats caused significant intrauterine growth retardation and decreases in the number of live pups (*34*). Some studies suggest that effects of transplacental B[*a*]P administration on fetal weight may persist into the postnatal period. After oral administration of B[*a*]P to pregnant CD-1 mice, pup weight was significantly depressed at day 20 and day 42 postnatal examination (*32*). The B[*a*]P diol-epoxide (BPDE) is a potent embryotoxin and teratogen, causing 100% malformations in Swiss mice pups following embryonal injection of BPDE, compared to 21% malformation in controls receiving vehicle-only injections (*31*).

There is evidence in humans that PAH, fine particles, and other pollutants from burning of coal may adversely affect birth outcomes. There was an excess prevalence of low birth weight and premature births in a polluted district of the Czech Republic (Teplice) compared to Prachatice, where pollution was low (*37*). These adverse effects were more common in infants conceived in the winter months and whose mothers were smokers (*21*). As mentioned, our research suggests that PAH in air pollution adversely affect birth weight, length, and especially head circumference (*27*). A possible mechanism for the latter is the induction of apoptosis following DNA damage by PAH.

PAH are widespread pollutants commonly found in ambient air, as well as workplace air, food, and drinking water (*38*). Incomplete combustion of gasoline and diesel fuels by transportation sources, and burning of coal and oil for industrial purposes and residential heating are the major sources of ambient PAH. ETS and home cooking and heating are the most significant indoor sources of airborne PAH (*39–41*) . The PAH with five or more rings (e.g., B[*a*]P) occur predominantly in the particulate phase, associated with deeply respirable particles smaller than 2 μm in diameter (*42*). Most studies that include both winter and summer data have found that PAH concentrations are higher during the winter (*43–45*). In addition to its occurrence in ambient air, B[*a*]P is a constituent of mainstream cigarette smoke (20–40 ng/cigarette) and sidestream smoke (68–136 ng/cigarette in undiluted smoke) (*46*). Drinking water intake is a less important source, generally less than 0.1 ng/L (*47*). Diet can be an important source of B[*a*]P (*47*), which is present in a wide variety of foods including charbroiled, grilled or smoked meat and fish (*48,49*).

Toxicity and Occurrence of Particulate Matter

The effects of PM on the developing fetus and infant have not been adequately studied. A research project in the Czech Republic found that during winter inversions in the more polluted area of Teplice, fine particles were dominated by acidic sulfates, organic compounds, and toxic trace elements—largely from the combustion of coal. There was an excess prevalence of low birth weights, premature births, and neurobehavioral problems in Teplice, compared to a less polluted area (*21*). A study in Beijing found an association between maternal exposure to total suspended particulate (TSP) and sulfur dioxide during the third trimester and decreased birthweight (*50*). Also in Beijing, preterm delivery was associated with TSP and sulfur dioxide (*51*). Finally, in the United States, postneonatal infant mortality has been associated with PM (*52*).

Combustion of coal, oil, gasoline, diesel and wood are among the major sources of respirable PM in urban air (*53*). However, individual exposure depends on particles encountered both indoors and outdoors, with indoor exposures being of prime concern because the vast majority of time is spent indoors. Infiltration of ambient respirable particulates into interior spaces occurs readily because of the low mass and long atmospheric residence times of tiny suspended particles (*54,55*). In homes with no smokers, indoor levels are typically similar to those measured outdoors (*56*). However, the contribution of smoking to indoor $PM_{2.5}$ (diameter < 2.5 μm) concentrations can be substantial, depending on numbers of cigarettes smoked. Other important indoor sources include cooking fumes, resuspended dust from carpets, furniture, and clothes, and emissions from stoves and kerosene heaters (*57,58*). $PM_{2.5}$ mass concentrations represent a heterogeneous mix of compounds, including sulfates, nitrates, elemental carbon, organic compounds including PAH, and metals including lead, cadmium, nickel, copper, vanadian, and zinc (*53*). In particular, there is a growing concern about the health effects of emissions from diesel exhaust. Diesel vehicles emit 30–100 times more particles than gasoline engines with contemporary emission-control devices (*59*). The relatively small size of the diesel exhaust particles (mass median diameter of 0.05–0.3 μm) makes them readily respirable (*60*). The particulate fraction is mainly formed of elemental carbon particles with organic compounds such as PAH and other carcinogens adsorbed on their surface.

Toxicity and Occurrence of ETS

ETS is an important source of PAH and appears to be an independent risk factor for fetal and child growth impairment capable of acting via non-PAH constituents. In addition to PAH, tobacco smoke contains carbon monoxide and a host of other developmental toxicants. ETS is a cause of low birth weight (LBW) and deficits in birth weight and height that persist into infancy and childhood. Prenatal exposure to maternal tobacco smoke has been shown to affect fetal growth and cognitive functioning at age 3 years (*28,61*). The effect of prenatal exposure to the mother's ETS on cognitive development has not been adequately studied. Paternal smoking has been associated with a reduction of 88 g in birth weight (*62*). The effect of postnatal ETS exposure is not clear (*63*); however, one study reported that reduced height was associated with postnatal parental smoking in the home, regardless of whether the mother smoked during pregnancy and regardless of which parent smoked (*64*). The effect of smoking on birth weight is thought to result in part from a reduction in blood supply to the fetus via vasoconstriction of umbilical vessels and an increase in fetal carboxyhemoglobin levels.

Several epidemiologic studies also indicate that exposure to ETS *in utero* or in childhood increases lifetime risk of cancer (*46,65–67*). An estimated 17% of lung cancers among nonsmokers has been attributed to high levels of ETS exposure during early childhood and adolescence (*66*).

Individual ETS exposure varies widely, depending on the number of smokers, amount smoked, amount of time spent indoors with smokers, and ventilation characteristics of the home. As discussed in the following section, ETS exposure in Poland is high (*68,69*). Similarly, ETS is a common urban pollutant in the United States, as evidenced by a recent finding of elevated cotinine levels in 80% of inner-city children sampled (*70*).

Molecular Epidemiology and Biomarkers

Most of the research to date has lacked adequate exposure and dosimetry data to forge causal links between common environmental factors and health effects in the young. In this regard, molecular epidemiology using biomarkers can be a valuable tool in defining environment–susceptibility relationships when used in conjunction with sound monitoring and epidemiologic methodologies (*23,71–76*). The following biomarkers were used in our research in Poland to assess the effects of *in utero* exposure and genetic susceptibility:

Plasma cotinine. Cotinine is a useful marker of exposure to tobacco smoke, with a half-life of 15–40 hr (*77,78*). A number of studies have concluded that cotinine is the most valid biologic indicator of active or passive exposure over the previous several days (*79–81*). A single determination also gives a good impression of steady-state levels in people having stable smoking habits (*79*). Cotinine readily crosses the placenta and is detectable in fetal samples (*15,82*). Cotinine levels have been used as an internal dosimeter in several studies of nonsmoking pregnant women to evaluate the effects of ETS exposure on fetal development. We and others have seen a significant association between ETS exposure during pregnancy and cotinine levels in maternal and infant samples collected at delivery (*83,84*). Significant decrements in birth weight in cotinine-determined ETS exposed compared to nonexposed infants were seen in some (*85,86*) but not all studies (*87*).

PAH–DNA adduct levels in white blood cells. Because DNA adducts are a biomarker that reflects individual variation in metabolism as well as repair of DNA damage, they provide an informative individual biologic dosimeter of specific genotoxic pollutants, supplementing monitoring of external exposures (*88–90*). With respect to developmental effects, our observation of a highly significant inverse correlation between decreased head circumference at birth and increased PAH–DNA binding (*27*) is consistent with other evidence that the developing CNS is particularly sensitive to DNA-damaging agents (*91–93*) and may respond by activating apoptotic pathways (*27*). Thus, although within a development study, we consider adducts to be primarily a dosimeter of environmental PAH, they may also reflect a mechanism of toxicity. In experimental studies, there is a high correlation between DNA adduct formation and carcinogenicity for a series of mutagens/carcinogens, including B[*a*]P (*89,90,94*). Supporting the use of peripheral blood cells as a surrogate for target tissue, experimental and human studies indicate comparable levels of DNA adducts across many tissues, including peripheral blood (*88,95*).

Cytochrome P4501A1 genotype and induction. Genetic differences in detoxification capabilities may modulate PAH-induced carcinogenesis (*96*). CYP1A1 is an inducible enzyme system that catalyzes the biotransformation of PAH to phenolic products and epoxides (*97,98*). These can be further biotransformed by epoxide hydrolase and other enzymes to reactive metabolites capable of binding to DNA (*99*). A *Msp*I restriction fragment length polymorphism (RFLP) has been identified in the 3′ noncoding region of the *CYP1A1* gene (the *CYP1A1 Msp*I RFLP). It segregates in linkage disequilibrium with a polymorphism in exon 7 that results in an Ile → Val substitution in the catalytic region. Both *CYP1A1* polymorphisms have been associated with lung cancer risk in some, but not all, studies (*100–106*).

CYP1A1 is inducible in human placental and other tissues. CYP1A1 mRNA reflects gene induction, whereas aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufun *O*-deethylase (EROD) are measures of enzyme activity. In human lung, a significant correlation has been seen between AHH activity and carcinogen–DNA adduct levels, including those formed by B[*a*]P, a representative PAH (*107,108*). In human placenta, induction of AHH and EROD activity as a result of maternal smoking has been well documented (*98,109–111*). One study found a high correlation between AHH activity and the formation of the procarcinogenic B[*a*]P-7,8-diol in human placental tissue (*110*); others did not see a high correlation between measures of CYP1A1 activity and BPDE–DNA adduct levels (*112,113*). Evidence suggests that CYP1A1 activity in the placenta reduces transfer of PAH to the fetus (*114,115*).

Glutathione S-transferase M1. Glutathione *S*-transferase M1 (GSTM1) codes for an enzyme involved in the detoxification of PAH via conjugation of activated metabolites with glutathione. An estimated 30–60% of populations are deleted at this locus (*116*). The GSTM1 null genotype has been associated with increased risk of lung cancer (*88,117–119*), albeit not consistently (*120–122*). Previous evaluations of the association between the *CYP1A1* and GSTM1 polymorphisms and carcinogen–DNA adduct levels have provided conflicting results (*123–127*).

Krakow, Poland: A Unique Opportunity for Research

The ethnically homogeneous population in Krakow, Poland, provides a valuable model for the evaluation of the health effects of ambient and indoor air pollution (PAH, PM, and ETS). The ambient air in the industrialized city of Krakow contains relatively high concentrations of PAH, PM and other environmental pollutants compared to other areas of the country, with the exception of Silesia (*128*). The pollution is attributable to transportation sources, industrial sources, and coal-burning furnaces, and is heaviest in the older, central section of the city (*129,130*). During the last 10 years, sulfur dioxide and PM concentrations have decreased as a result of the reduced productivity of the old heavy industry within and around the city, and the introduction of a gas-operated heating system in the city (covering approximately 30% of the homes). However, the levels of PAH, PM, and heavy metals continue to be high in the central city because of increasing traffic (especially diesel) and continued coal burning by industrial and residential sources. Krakow also receives air pollution from the Upper Silesia coal region (*130*).

PAH are major toxic constituents of air pollution in Krakow. According to air monitoring data provided by the Office of Environmental Protection, which operates monitoring stations in Krakow, in 1996, annual average concentrations of B[*a*]P, a representative PAH, were 2-fold higher in Srodmiescie, the most polluted, central area of Krakow (annual average 13.3 ng/m^3, 22.4 in winter; 4.6 in summer) than in Krowodrza, the cleanest area (annual mean of 6.0 ng/m^3; 11.2 winter; 0.9 summer). There was an almost 5-fold seasonal variation in PAH concentrations within Srodmiescie and 12-fold variation within Krododrza.

Levels of respirable particulates are also high in Krakow. During 1991, the year prior to the birth of the infants in our study, annual average levels of respirable particulates (< 10 mm in diameter) were 78 µg/m^3 in the high pollution area and 38 µg/m^3 in the low-pollution area of the city. As seen from Figure 1, particulate pollution has been reduced since 1991 but still remains high and continues to vary by approximately 2-fold between Srodmiescie and Krowodrza. There is also significant seasonal variability in particulate levels.

ETS exposure is high in Poland. A 1992 study of nonsmoking Polish women found that the vast majority were exposed to ETS either at home or the workplace and that urinary cotinine was detected in 92% of the nonsmoking women sampled (*68*). In our study, 58% of the nonsmoking women from Krakow reported ETS exposures, and among these nonsmoking women, cotinine was detected in 42% of the blood samples collected from their infants at birth (*11*).

Rates of infant mortality and LBW have increased significantly in Poland between 1985 and 1995 and are higher in the industrialized, heavily contaminated regions of the country than in the rural areas (*131–133*). Ambient air pollution has been suggested as a possible causal agent (*128,134*). The highest levels of infant mortality are generally found in the provinces with the most severe ambient pollution levels. Preliminary data suggest a correlation between infant mortality rates and several measures of ambient air pollution, including dustfall and B[*a*]P (*128*). A 1990 study of placentas from 1,000 nonsmoking mothers in Krakow and other polluted areas in Poland found a high prevalence of gross

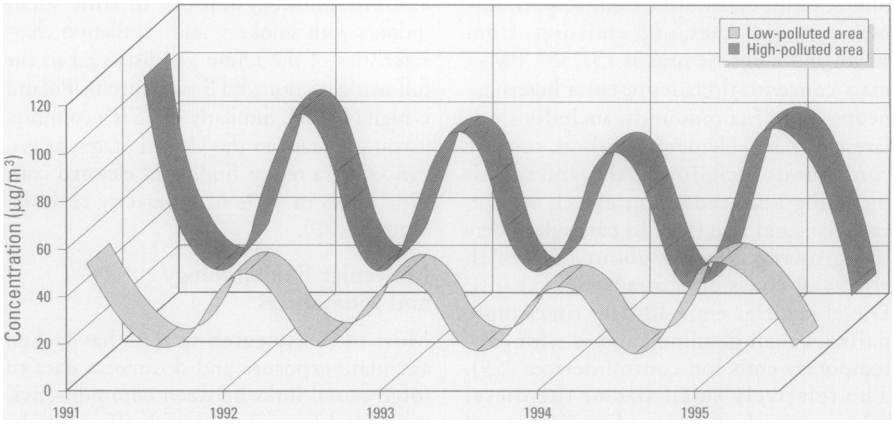

Figure 1. Levels of respirable particulates 1991–1995 (winter/summer) in high-polluted (Srodmiescie) and low-polluted (Krowodrza) areas of the Krakow.

structural changes in placentas from central Krakow that could have contributed to growth impairment of the fetuses (*128*). An ecologic analysis compared birth weights and rates of stillbirths for cities and rural villages of Poland from 1975 to 1988 (*135*). The incidence of LBW (< 2,500 g) and stillbirths was higher in the cities compared to the villages. The authors concluded that this finding could not be attributed to differences in diet or parental stature, and that it was probably due to the negative effects of urbanization and industrialization, including environmental pollution (*135*). A more recent case–control study found that urban residency was also a significant risk factor for small-for-gestational-age infants, controlling for other potential confounders (*136*).

In addition, there is evidence of "hot spots" for cancer mortality that correspond to geographical areas within Poland that are characterized by heavy industrialization and high pollution (*137*). Krakow has an excess of lung cancer deaths compared with Poland overall. Jedrychowski and colleagues have reported a statistically significant association between air pollution in Krakow and lung cancer risk among men (*129*).

A Molecular Epidemiologic Study in Polish Newborns and Mothers

Major Aims

- Evaluate the relationship between ambient exposures and PAH–DNA adducts.
- Assess the effect of genetic/metabolic markers (placental CYP1A1 mRNA and EROD activity), GSTM1 genotype, and CYP1A1 *Msp*I polymorphism on adduct levels.
- Compare DNA adducts in maternal and fetal/newborn samples to assess possible differences in response and susceptibility.
- Assess the association between infant PAH–DNA adduct levels and birth outcomes (birth weight, length, and head circumference) after controlling for gestational age.

Study Design

The study included 320 subjects: 70 mother/newborn pairs from Krakow and 90 mother/newborn pairs from Limanowa, a small town in a rural agricultural district of Poland with lower ambient pollution but heavier use of coal for residential heating (*27,138*). Enrollment was restricted to women who had resided in Krakow or Limanowa for at least 1 year and was limited to vaginal deliveries. Enrollment alternated on a biweekly basis between Krakow and Limanowa in the winter of 1992 to control for monthly variations in pollutant levels. Immediately after delivery, samples of umbilical cord blood and placenta were collected. A maternal blood sample was collected within 2 days postpartum. Samples were processed and stored as described (*138*).

A questionnaire administered to the mother within 2 days postpartum included information on smoking, residential and employment histories, use of coal stoves for residential heating, and other environmental exposures. Subjects were asked to estimate the average number of weekly servings of specific foods consumed during pregnancy, such as smoked meats, cheese, and fish, as potentially high dietary sources of PAH. In addition, subjects were asked about exposure to sources of PAH either at home or in the workplace (including coal stoves and charcoal, tar roofing material, etc.) as well as exposure to pesticides and other organic chemicals as potential inducers of CYP1A1. All interviews were conducted by two trained interviewers from the Department of Epidemiology and Preventive Medicine, Jagellonian University (Krakow). Coded interview data were sent to Columbia University (New York, NY). Assessment of smoking status was based on questionnaire data and plasma cotinine (*138*).

Daily ambient monitoring data for Krakow (1990–1992) were provided by the Division of National Sanitary Inspection (Krakow; 15 monitoring stations) and by the U.S. Environmental Protection Agency (5 monitoring stations). Each Krakow woman's exposure to ambient particulates, as a proxy for air pollution, was estimated by taking the average of PM_{10} measurements (in micrograms per cubic meter) reported at the monitoring station closest to her residence for each of the past 2 years and the month prior to her delivery date. Ambient particulate data were available for 69/70 subjects from Krakow. As there was only one ambient monitoring station in Limanowa, individual ambient exposures could not be estimated for Limanowa subjects.

As a first step in analyzing the effects of ambient air pollution on biomarker levels (*138*), the difference in biomarker levels between residents of Krakow versus Limanowa was determined. Then, Krakow subjects were trichotomized into low-, medium-, and high-pollution groups based on the ambient PM_{10} level at their place of residence. The difference in biomarker levels across pollution groups was determined for all Krakow subjects and for Krakow subjects not employed away from home during pregnancy. Estimates of exposure for the latter group were considered more reliable as unemployed women spend more of their time at their place of residence.

For statistical analyses, biomarkers were log-transformed as needed to stabilize the variance and obtain a more symmetrical distribution. Associations were initially evaluated by chi-square and Student's *t*-test followed by multivariate analyses. The regression models included cigarette smoke exposure, place of residence (Krakow or Limanowa), ambient pollution group (high, middle, low; Krakow only), average number of servings per week during pregnancy of foods high in PAH (smoked meat, cheese, and fish), use of coal stoves for residential heating (yes/no), and home/occupational exposures to PAH and other organics.

Analysis of the relationship between ambient exposures and adducts. After controlling for smoking status and other potential confounders, overall, there was no difference in the mean PAH–DNA adduct levels measured by enzyme-linked immunosorbent assay in mothers and infants from Krakow compared to those from Limanowa, possibly because of higher indoor air concentrations of PAH from coal burning in Limanowa. Although coal use (yes/no) was controlled in the multivariate analyses, more precise measures of use (duration and frequency of use of coal stoves) were not obtained. When we restricted analysis to the noncoal users, adduct levels in maternal white blood cells (WBC) were about 2-fold higher in Krakow compared to Limanowa ($p = 0.03$, Student's *t*-test).

In Krakow, among subjects for whom the exposure estimations can be considered most reliable (those women not employed away from the home) a dose–response relationship was seen between PAH–DNA adduct levels in maternal and infant WBC (but not placental tissue) and increasing ambient PM_{10} pollution at the woman's residence ($p \leq 0.05$, controlling for smoking status and other potential confounders, Figure 2) (*11*). The trend for increasing maternal WBC adduct levels with increasing ambient air pollution groups was statistically significant ($p = 0.02$). Adduct levels were significantly increased in women and newborns residing in the high- compared to low-pollution area ($p \leq 0.05$).

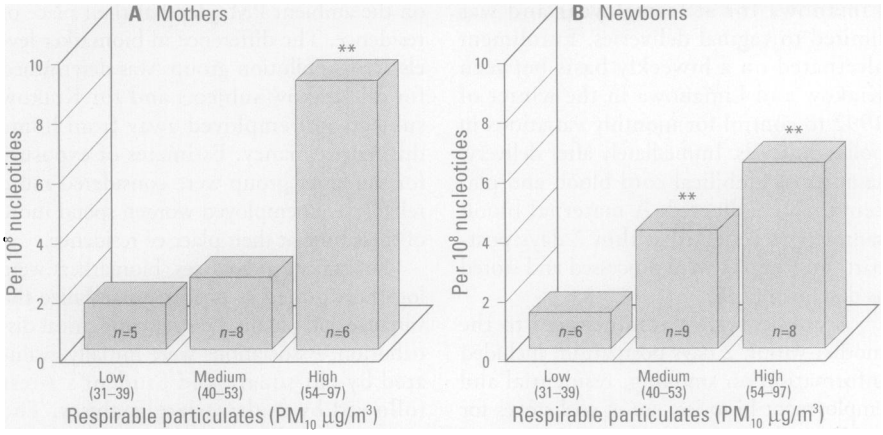

Figure 2. WBC PAH–DNA adducts by level of air pollution in Krakow subjects not employed outside the home. Geometric means adjusted by smoking status, dietary PAH, use of coal stoves for residential heating, and home/occupational exposures to PAH and other organics. $^{**}p \leq 0.05$ compared to low-pollution group.

Maternal WBC PAH–DNA adduct levels were significantly associated with active cigarette smoking status ($p < 0.01$) and, among nonsmokers, were significantly higher in subjects reporting ETS exposure compared to those reporting no ETS exposure ($p = 0.01$) (*11*).

There was substantial interindividual variation (30 to 40-fold) in adduct levels among the women and infants. These results are consistent with those from a Northern Bohemian study in which PAH–DNA adducts in women's WBC varied by 26-fold and were highly correlated with individual personal exposures to PAH monitored over a 24-hr period (*139*). The authors noted that the personal exposures were generally relatively low, comparable to those found in other urban areas of North America and Europe. In spite of this, there was a sufficiently high interindividual variability in personal exposures to PAH to demonstrate a significant correlation between individual PAH exposure and DNA adducts ($r = 0.541$, $p < 0.016$).

Analysis of the relationship between genetic/metabolic factors, exposure and biomarkers. A significant association between ambient pollution at the women's place of residence within Krakow and placental CYP1A1 mRNA was seen among subjects not employed away from home ($p < 0.05$) (*138*). Placental CYP1A1 mRNA was significantly associated with active cigarette smoking status and were highly correlated with infant plasma cotinine levels ($p < 0.001$) (*11,138*). Ex-smokers also had significantly increased placental CYP1A1 mRNA levels compared to nonsmokers. PAH–DNA adduct levels in both placental tissue and infant WBC were not significantly associated with placental CYP1A1 MRNA.

GSTM1 is expressed rarely in fetal tissues, and then only at low levels; therefore, infant *GSTM1* genotype was not analyzed. Genotyping at the GSTM1 locus was completed for 143/160 maternal samples. Of these, 72 (50%) were homozygous deleted (GSTM1–/–). The remaining 71 (50%) of the women had one or two copies of the gene (GSTM1+/+, +/–).

Determination of the CYP1A1 *Msp*I RFLP was completed for 142/160 maternal samples. Of these, 24 (17%) were heterozygous for the restriction site (CYP1A1 *Msp*I+/–) and 118 (83%) did not have the restriction site (CYP1A1 *Msp*I–/–). None of the women were homozygous for the restriction site (CYP1A1 *Msp*I+/+). Determination of the CYP1A1 *Msp*I RFLP was completed on 158/160 infants (140/160 umbilical cord DNA samples and 149/160 placental DNA samples). Of these, 29 (18%) were heterozygous (CYP1A1 *Msp*I–/+) and 3 (2%) were homozygous (CYP1A1 *Msp*I+/+). The remaining 126 (80%) of the infants did not have the restriction site (CYP1A1 *Msp*I–/–). As in other studies, homozygotes and heterozygotes were combined in the analysis.

Maternal WBC adduct levels were not associated with either the CYP1A1 *Msp*I RFLP or GSTM1 genotype, before or after controlling for potential confounders (*11*), nor was there an interaction between the genotypes and exposure on maternal adduct levels. However, adjusting for potential confounders, PAH–DNA adduct levels in both placental tissue and infant WBC were 1.7-fold higher in infants who were heterozygous or homozygous for the CYP1A1 *Msp*I RFLP than in infants without the restriction site. The difference was significant for placental tissue ($p < 0.01$, $n = 158$) and of borderline significance for infant WBC ($p = 0.06$, $n = 135$), controlling for potential confounders (*11,69*). CYP1A1 genotype was not correlated with CYP1A1 enzyme (*138*). The effect of the CYP1A1 genotype on adduct formation was greater than that of CYP1A1 activity.

The differing relationship between *CYP1A1* and adducts in the women and the newborns may result in part from reduced detoxification capabilities via phase II enzymes in fetal tissues (*1,3*) rendering the fetus more susceptible to the effects of the CYP1A1 genotype. The results suggest that a subset of infants (those with the *CYP1A1* polymorphism) may be at heightened risk from environmental exposures to PAH.

Comparison of the biomarkers in mother/infant pairs. Of the 160 mothers and infants in the study, 112 pairs (224 subjects) had adequate amounts of DNA for adduct analyses. Comparison of PAH–DNA adduct levels in these 112 paired maternal and infant WBC samples showed that adduct levels were higher in infants than in mothers (7.9 ± 0.93; vs 5.9 ± 0.77 per 10^8 nucleotides). Although the difference was not significant ($p = 0.13$, Wilcoxon matched-pairs signed-ranks test), the results are noteworthy, as the transplacental dose of PAH is estimated to be 10-fold lower in the fetus compared to the mother. Adduct levels in placental tissue were also significantly higher than those in paired maternal WBC samples (8.2 ± 0.57 versus 6.4 ± 0.81 per 10^8 nucleotides, $p < 0.01$, Wilcoxon signed-ranks test).

Analysis of the association between PAH–DNA adduct levels and fetal development. Compared to newborns from Limanowa, Krakow newborns had lower mean birth weight and significantly lower birth length and head circumference, before and after controlling for maternal height, age, socioeconomic status, history of LBW, alcohol consumption, gestational age, gender of the newborn and plasma cotinine (*27*). The combination of known risk factors and PAH–DNA adducts did not explain the observed difference in birth outcomes between Krakow and Limanowa. It is possible that unmeasured factors of urbanization, including constituents of air pollution other than PAH, are contributing to fetal growth impairment in Krakow.

Table 1 presents differences in birth outcomes for newborns with high versus

Table 1. Difference in birth outcomes for Polish newborns with high (> median) compared to low (< median) leukocyte PAH–DNA adduct levels.[a]

	Birth weight		Birth length		Head circumference	
	Difference (g)	*p*-Value	Difference (cm)	*p*-Value	Difference (cm)	*p*-value
Krakow newborns ($n = 58$)	−205	0.11	−1.8	0.02	−0.9	0.05
Limanowa newborns ($n = 77$)	−129	0.16	−0.8	0.17	−1.2	0.0004
All newborns (Krakow + Limanowa)	−147	0.05	−1.1	0.02	−0.9	0.0005

[a]Controlling for maternal height, age, socioeconomic status (education level), history of low birth weight, maternal alcohol consumption, gestational age (weeks), gender of the newborn, and plasma cotinine (ng/mL). Median adduct level 3.85/10^8 nucleotides.

low PAH–DNA adducts (*27*). In both Krakow and Limanowa groups analyzed separately, all three measures (birth weight, length, and head circumference) were decreased in newborns with high adduct levels, although the differences were not significant in all cases. Among Krakow newborns, those with elevated PAH–DNA adducts had significantly decreased birth length (by 1.8 cm, $p = 0.02$) and head circumference (by 0.9 cm, $p = 0.05$). Among Limanowa newborns, those with elevated adducts had significantly decreased head circumference (by 1.2 cm, $p = 0.0004$). Considering all the newborns combined (Krakow and Limanowa), those with high PAH–DNA adducts had significantly lower birth weight ($p = 0.05$), birth length ($p = 0.02$), and head circumference ($p = 0.0005$) than newborns with low adducts (*27*).

After combining the two groups and removing current smokers in order to investigate the effects of PAH in the absence of active smoking by the mother, all three measures of fetal development remained significantly lower among those with high versus low adducts ($p \leq 0.03$). To remove the effect of both active and passive smoking, analyses were further restricted to the 53 newborns of nonsmokers without detectable plasma cotinine. The newborns with elevated adducts had decreased birth weight (by 217 g, $p = 0.1$) and length (by 0.8 cm, $p > 0.2$) and significantly decreased head circumference (by 1.3 cm, $p = 0.006$). When PAH–DNA adducts among all the newborns were included in the regression models as a continuous variable, they were inversely, although not significantly, correlated with birth weight and length. Adducts were inversely correlated with head circumference both before ($p = 0.006$) and after controlling for birth weight ($p = 0.003$), suggesting asymmetrical growth retardation (*27*).

The results do not imply that developmental damage is necessarily mediated by DNA binding, although that is one possible mechanism. Here the extent of DNA binding by PAH in newborn leukocytes has been used as a dosimeter of PAH that have reached the fetus. Neither the mechanisms by which PAH exert developmental toxicity nor the target sites have been identified (*27*). Indeed, it is possible that PAH act by more than one mechanism. For example, it has been hypothesized that B[*a*]P exposure may interfere with uterine growth during pregnancy due to its antiestrogenic effects, thereby disrupting the endocrine system (*29*). Similar to PCBs, which are associated with deficits in fetal growth and IQ (*140,141*), PAH bind to the human Ah receptor to induce P450 enzymes (*142*). Additionally, the developing CNS appears particularly sensitive to DNA-damaging agents (*91*) and may respond by activating apoptotic pathways (*92*). For example, in humans, fetal microcephaly has been seen following exposure to ionizing radiation (*143*) and anticonvulsant drugs (*144*). Risk from anticonvulsants was most pronounced in infants deficient in enzymes that detoxify the DNA-binding intermediate (*145,146*). It is of interest that, in this Polish cohort, PAH–DNA adduct levels were most strongly associated with reduction in head circumference, and the data were suggestive of asymmetrical growth retardation related to DNA binding.

Analyses of the Relationship between Cotinine and Biomarkers/Development

Maternal and infant cotinine levels were increased significantly by active cigarette smoking of the mother ($p < 0.0001$) (*83*). Additionally, for women who reported not smoking during pregnancy, cotinine levels in both mothers and infants were significantly increased with ETS exposure ($p < 0.01$). An inverse correlation was seen between newborn plasma cotinine (nanograms per milliliter) and birth weight ($p = 0.0001$) and birth length ($p = 0.003$), controlling for place of residence and other potential confounders. The effect of cotinine on outcomes was independent of that of PAH–DNA adducts (*27*). Further, cotinine provided a better fit (i.e., explained more of the variance in birth weight) than other measures of maternal smoking derived from the questionnaire, none of which were significantly associated with birth weight (*83*). Cotinine levels in infant samples were higher than in paired maternal samples, a difference that was significant both for the total cohort (14.2 ± 2.8 vs 8.3 ± 2.0, $p < 0.0001$) and after analyses were restricted to mother/infant pairs for whom blood samples were collected within 6 hr of one another (average of 0.9 ± 1.2 hr SD; 10.6 ± 3.5 vs 8.0 ± 2.6, $p = 0.0001$).

Summary

In conclusion, these results indicate that there is significant transplacental transfer of PAH and ETS constituents from mother to fetus; that maternal and infant WBC PAH–DNA adduct levels were increased with environmental exposure to PAH from ambient pollution; and that plasma cotinine is increased by ETS. The finding of higher adduct levels in the infant compared to the mother suggests increased susceptibility of the developing fetus to DNA damage, whereas the finding of higher cotinine levels suggests reduced capability of the developing fetus to detoxify and clear cigarette smoke constituents. The study also provided the first molecular evidence that transplacental PAH exposure to the fetus may be compromising fetal development. Although these findings implicate DNA damage as the mechanism, it should be noted that, like PCB, PAH are capable of inducing P450s and disrupting the endocrine system. A follow-up study is needed to confirm whether IQ/cognitive function is decreased among children with smaller head circumference associated with exposure. If confirmed, these findings could have significant public health implications, as a number of studies have found that reduction of head circumference at birth correlates with lower IQ as well as poorer cognitive functioning and school performance in childhood (*20,25,26*).

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